user manual

DNA/Protein Labeling, Hybridization & Detection

DyNA Quant 120 Capillary Adaptor Kit

an accessory to the DyNA Quant 200

um 80-6232-00 DQ120-IM/Rev B0/8-99

Amersham Biosciences

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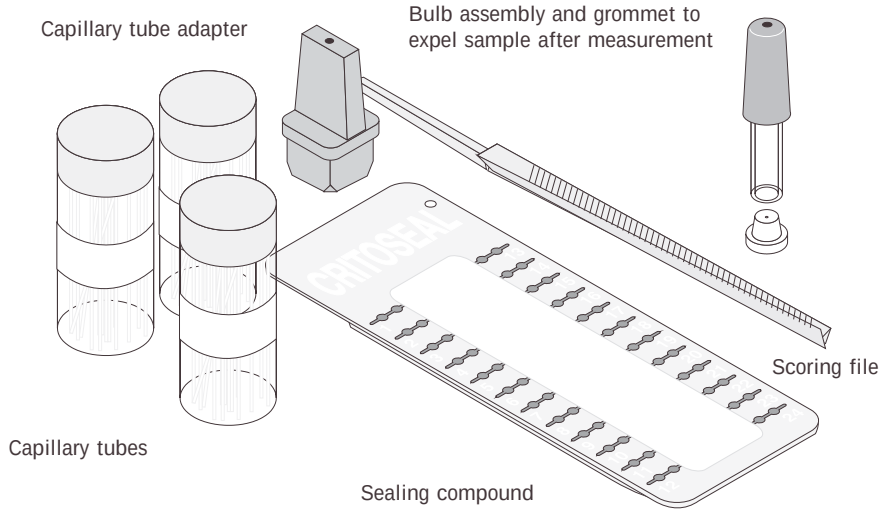
Capillary Adaptor Kit Function

Use the DyNAQuant™ 120 Capillary Adaptor Kit for microvolume (10-100 µl) quantitation of DNA or enzyme activity. With this adaptor, sample can be easily recovered for further processing. Capillaries are disposable and thus eliminate cross contamination.

The kit includes 20 each of 10-, 50-, and 100-µl capillary tubes, one capillary tube adaptor (which fits most fluorometers), one scoring file, one bulb assembly and silicone grommet (to hold the capillary), and one pad of sealing compound.

Unpacking

Unwrap all packages carefully and compare contents with the packing list, making sure all items arrived. If any part is missing, contact your local sales office. Inspect all components for damage that may have occurred in transit. If any part appears damaged, contact the carrier immediately. Be sure to keep all packing material for damage claims or for repacking should it become necessary to return an item.



Important

- Hoechst 33258 dye is a possible carcinogen and possible mutagen.

  Wear gloves when handling. Wear a mask when weighing. Disposal must comply with all applicable regulations. Never dispose of by pouring into a drain.

- Because these measurements are highly sensitive, take extra steps to assure clean materials and reagents:

  Use sterile pipette tips and sample tubes. Make TNE buffer with double-distilled sterile water. Filter buffers and water before adding H 33258. Use only highly pure chemicals. Wear gloves

Fluorescence measurement

DyNA Quant 200

Bisbenzimide, commonly known as Hoechst 33258 (H 33258) dye, exhibits changes in fluorescence characteristics in the presence of DNA that allow accurate DNA quantitation. In the absence of DNA, the fluorescence excitation spectrum of H 33258 peaks at 356 nm and the emission spectrum peaks weakly at 492 nm. When H 33258 binds to DNA, these peaks shift to 365 nm ex and 458 nm em. In the cuvette well, the sample is exposed to filtered light (365 ± 7 nm) from a mercury lamp. This light excites the DNA-dye complex, causing light that peaks at 458 nm to be emitted. An emission filter in front of the the photodetector allows only fluorescence at 460 nm, ±15 nm, to register. Thus the measured fluorescence is a direct indicator of the DNA concentration.

Measurement considerations

Dilute DNA standard and sample in 1X TE to ensure the optimal pH value (7.4) and maximum signal to noise ratio.

The dye concentration for the capillary adaptor assay is necessarily higher than the dye concentration for the 2 ml cuvette assay because the same amount of DNA is in a much smaller volume of assay solution. Enough dye must be present to preserve the linear relationship between the amount of DNA and the fluorescence measured.

Note Using smaller volumes does not increase the sensitivity of the assay.

Note The blank, standard, and sample solutions all must contain the same final dye concentration at the same pH and all must have similar ionic strength.

The DNA reference standard (1 mg/ml solution of calf thymus DNA) serves both to provide a one-point reference for determining the DNA concentration of unknown DNA samples and as a standard to evaluate the performance of the fluorometer.

Generate a standard concentration curve for maximum accuracy. Analyse the results by graphing the sample concentration (x) vs the averaged reading (y). As long as the graph is linear you can expect accurate values for unknown samples within the range of the standard curve. (Slight variations are most commonly due to pipetting variability.)

Method overview

1 Turn on the fluorometer lamp at least 15 minutes before using.

2 Prepare stock solutions. Prepare all blank, standard, and sample solutions in microfuge tubes with lids.

3 Zero and calibrate the instrument.

Optional: Measure a series of standard concentrations and plot a concentration curve that spans the expected sample concentration range. Analyze the results by graphing or determining the least squares fit equation of the line.

4 Measure the fluorescence of the unknown DNA sample. If you constructed a concentration curve, correlate the measurements to the curve.

Solutions

Important: Refer to the material safety data sheet (MSDS) accompanying each chemical for detailed handling and safety information.

Hoechst 33258 stock solution (1mg/ml)

Hoechst 33258	10 mg
Distilled filtered water	10 ml

Do not filter. Store in an amber bottle at 4 °C for up to 6 months.

1M Tris-Cl, pH 7.4 (100 ml)

Tris base [Tris (hydroxymethyl) aminomethane], MW=121.14	12.11 g
dd H_20	80 ml

Adjust pH to7.4 with concentrated HCl. Let solution cool to room temperature before final addition of HCl to pH 8.0. Adjust final volume to 100 ml.with distilled water. Autoclave.

5M NaCl (100 ml)

NaCl	29.22 g

Add distilled water to 100 ml and autoclave.

Note Disodium EDTA does not go into solution until pH approaches 8.0.

0.5M EDTA (disodium ethylenediaminetetracetate, pH 8.0, 100 ml)

EDTA	18.61 g
dd H20	80 ml

Stir vigorously with magnetic stir bar. Titrate pH to approximately 8.0 with NaOH. Adjust final volume to 100 ml.with distilled water. Autoclave.

1X TE buffer stock solution (10 mM Tris-Cl, 1 mM EDTA, pH 7.4, 100 ml)

1 M Tris-Cl, pH 7.4	1 mL
0.5 M EDTA, pH 8.0	0.2 mL
dd H_20	98.8 mL

Adjust pH to 7.4 with concentrated HCl. Autoclave and store at room temperature for up to 3 months.

10X TNE buffer stock solution (100 mM Tris, 10 mM EDTA, 2 M NaCl, 100 ml)

1M Tris-Cl, pH 7.4	10 ml
0.5 M EDTA, pH 8.0	2 ml
5M NaCl	40 ml

Add distilled water to 100 ml. Adjust pH to 7.4 with concentrated HCl.Autoclave and store at 4 °C for up to 3 months.

Calf thymus DNA (100 µg/ml) (1:10 dilution of standard; may require further dilution)

100 µl calf thymus DNA standard, 1 mg/ml
100 µl 10X TNE
800 µl distilled water, filtered

Gently tap the tube to mix thoroughly. Store at 4°C for up to 3 months.

2X Capillary assay solution (20 µg/ml H 33258 in 1X TNE) pH 7.4

H 33258 stock solution	20 µl
10X TNE buffer stock solution	100 µl
Distilled filtered water	880 µl

Prepare fresh daily. Keep at room temperature. Do not filter once the dye is added.

Operating instructions

Setup

Since the DNA fluorescence assay is based on a relative measurement of emitted light, a calibration reference value must be established with a known DNA sample before the concentration of DNA in unknown samples can be determined. Once the initial reference value can be reliably reproduced, you can proceed to determine the concentrations of unknown samples, or determine assay linearity with standard concentration measurements. Generating a standard curve once every few weeks serves as a quality check on the standard, a reliability check on the instrument, and a consistency check on technique.

1 Turn on the fluorometer lamp at least 15 minutes before taking measurements so that the lamp has time to stabilize.

Note Switch the prompt off when measuring fluorescence with the capillary adaptor. (Select 2>Setup, 1>Prompt, and select 1>Off.)

2 Prepare stock solutions.

3 Select a capillary size according the desired DNA sample volume (see table below). You will need one "blank" to zero the instrument, one standard solution to calibrate the instrument (optional: plus a series of standard concentrations for a standard curve), and DNA samples.

Important All solutions to be measured must contain enough 10X TNE buffer stock so that the final concentration is no less than 1X TNE.

Prepare all solutions in microfuge tubes with lids. Keep lid closed between measurements to prevent evaporation.

Capillary size (µl)	DNA range (ng)	DNA solution (µl)	CAS (µl)
10	25–1500	5	5
50	10–1500	45	5
100	10–1500	95	5

Example

This example is for preparing solutions for 50 µl capillary tubes using a standard containing 1000 ng DNA in 50 µl.

Blank	45 µl 1X TNE buffer + 5 µl CAS
Standard	45 µl DNA + 5 µl CAS (for 1000 ng total DNA in 45 µl final: 10 µl 100 ng/µl DNA + 35 µl 1X TNE)
Sample	45 µl DNA sample in 1X TNE + 5 µl CAS

Zero the instrument

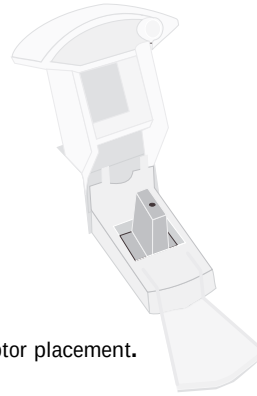
1 Fill a capillary with "blank" solution. Insert the capillary tube into the sample solution and allow the liquid to almost fill the tube. Tilt the larger 50- and 100-µl tubes into a horizontal position if necessary.

2 Seal the capillary tube. Hold the sealant pad vertically, press the tube no more than 2 mm into the sealant, and twist gently. Wipe the tube with tissue and inspect the seal for leaks.

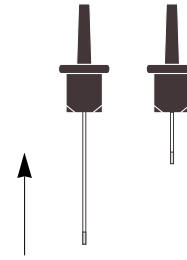
3 Insert the unsealed end of the blank into the adaptor. As you place the capillary and adaptor in the cuvette well, aim the capillary end of the assembly so that it fits into the depression in the bottom of the well. Gently push the adaptor into place.

Note The sealant, which fluoresces, must be completely seated in the depression to avoid erroneous readings.

4 Close the lid, and press <ZERO>.



Adaptor placement.



Capillary position before and after installing adaptor into the cuvette well.

Set a reference point with DNA standard

1 Fill a capillary with the appropriate DNA standard. (We suggest using a DNA concentration higher than the expected concentration of the unknown sample.)

Seal the capillary tube as in step 2 above.

2 Insert the capillary into the adaptor, position the adaptor in the cuvette well, close the lid, and press <CALIB>. Enter the actual concentration of the standard or enter a convenient value that will display a multiple of the actual DNA concentration. Press <ENTER>.

Example If your standard contains 100 ng DNA and you enter 25, each unit then corresponds to 0.25 ng DNA in the capillary, and the value displayed must be multiplied by 4.

3 If desired, measure a second capillary containing a standard solution to verify that results are reproducible.

One reference point is adequate to set the instrument. However, generating a standard concentration curve assures assay linearity in the range of interest.

An example of setting up a standard curve with a DNA range of 0 to 1500 ng for a 50-µl capillary tube follows, on page 9.

Construct a standard curve

1 Prepare a set of standards.

The following example applies to 50 µl capillary tubes containing up to 1500 ng total DNA. First mix the desired amount of DNA with 1X TNE to a volume of 45 µl. Use a 1:10 dilution of the 1 mg/ml DNA standard for a 100 ng/µl working solution. Then add 5 µl capillary assay solution.

Note For multiple measurements, multiply each amount by the number of readings desired.

Desired ng DNA per tube	0	300	600	900	1200	1500
µl DNA solution, 100 ng/µl (1:10 dilution)	0	3	6	9	12	15
1X TNE, to 45 µl total	45	42	39	36	33	30
Capillary assay solution, µl	5	5	5	5	5	5

2 Measure a sample for each concentration. If desired, measure a second sample and average the readings.

3 Plot the data or enter it into a math program to find the linear best fit equation of the line passing through the points. (See the DyNA Quant 200 instructions, Section 4 "Analyze the results" for more detail.)

Measure unknown DNA concentration

1 Fill the capillary with DNA sample solution and seal.

2 Insert the capillary into the adaptor and place the adaptor into the cuvette well. Close the lid and record the reading. Depending on how the instrument was calibrated, the display shows either the actual DNA concentration or a multiple of the concentration.

If the reading is above the linear range of the concentration curve, use less DNA and repeat the assay.

3 Determine the concentration from the standard concentration curve or calculate the concentration from the equation of the standard curve. (See the DyNA Quant 200 Instructions, Section 4 "Analyze the results" for more detail.)

To recover the sample for electrophoresis

1 Remove the capillary from the adaptor and fit the bulb on the capillary, taking care to not cover the hole at the top of the bulb.

2 Score the capillary tube with the file just above the sealing compound plug and carefully snap this section off.

3 Cover the opening in the bulb with your fingertip and squeeze gently to expel the sample into a tube.

Troubleshooting

Always be sure to:

- ✓ Operate the unit in a location isolated from equipment that radiates high-frequency electromagnetic interference.
- ✓ Operate the unit away from direct sunlight.
- ✓ Place the instrument so that the back vents are not obstructed.

Fluorescence values drift

- ✓ Assay solutions must be at ambient temperature for consistent readings. (Fluorescence decreases as temperature increases.)
- ✓ Protect fluorescent reagents and samples from light to prevent photobleaching (destruction of the fluorescent compound by light).
- ✓ Take readings immediately to prevent photobleaching.
- ✓ Assay solutions must be at pH 7.4.
- ✓ Adjust the salt concentration. For standard DNA, the concentration should be at least 200 mM NaCl in 1X TNE. For crude cell lysates, use 2 to 3 M NaCl in 1X TNE.

Wide fluctuations in fluorescence values

- ✓ Thoroughly mix the sample and assay solution.
- ✓ Use a micropipet accurate to at least 0.02 µl. If inconsistencies persist, dilute the sample and use larger aliquots.
- ✓ Take care that the DNA sample is completely suspended.
- ✓ Use only pure distilled and filtered (0.2 or 0.4 µm filter) water for all solutions.
- ✓ Filter the 1X TNE buffer to remove all particulates. Particulates may cause light to scatter, causing measurement fluctuations. (Filter the buffer **before** adding Hoechst dye because the dye binds to most membrane types.)
- ✓ Use as little sealing compound as possible. (Sealant is fluorescent and will cause erroneous readings if it enters the light path.)
- ✓ Wipe the capillary to remove fingerprints and liquids.

Readings negative or lower than expected

- ✓ Use freshly prepared assay solution at ambient temperature to set the zero and for all subsequent measurements.
- ✓ Extract ethidium bromide from DNA solutions because it interferes with the fluorescence of Hoechst dye.
- ✓ If expected DNA values are based on A_{260}/A_{280} readings, the sample may be contaminated with RNA, nucleotides, or protein, which are not detected by H 33258 fluorescence.

Crude cell lysates prepared with acid guanidinium thiocyanate-phenol solution

- ✓ Fluorescence of lysates prepared without an alkaline EDTA pretreatment is reduced by 70% compared to lysates with such pretreatment. Alkaline conditions allow formation of complexes between DNA and the dye. For a detailed protocol see: Rymaszewski, et al, (1990) Estimation of cellular DNA content in cell lysates suitable for RNA isolation. *Anal. Biochem*. 188: 91– 96.

Use the appropriate reference standard

- ✓ Use an ultra-pure grade DNA standard.
- ✓ Make sure to use a standard with a G-C content very similar to the sample DNA. Hoechst dye binds preferentially to A-T regions, so G-C content must be similar to ensure "equivalent" binding.
- ✓ Use a ssDNA standard for ssDNA samples. (Single-stranded DNA yields about half the fluorescence of an equal amount of double-stranded DNA.)
- ✓ Plasmid DNA standards should have the same conformation as the sample. Each form—supercoiled, relaxed, circular or linear—may have slightly different dye binding characteristics.

Readings higher than expected

- ✓ Fluorescence enhancement may result from high levels of detergents. Final SDS concentration should be below 0.01% and other detergents below 10 µg/ml (the final concentration of any detergent should be well below its critical micelle concentration.
- ✓ Use a reference standard with a G-C content very similar to your sample.
- ✓ Single-stranded genomic DNA yields about half the flu

Customer Service Information

Technical Service and Repair

Amersham Pharmacia Biotech offers complete technical support for all our products. If you have any questions about how to use this product, or would like to arrange to repair it, please call or fax your local Amersham Pharmacia Biotech representative.

Important: Request a copy of the Amersham Pharmacia Biotech "Contamination Clearance Certificate" before returning the item. No items can be accepted for servicing or return unless this form is properly completed.

Ordering Information

Basic Unit

DyNA Quant 200 Fluorometer. Includes DNA standard, Hoechst 33258 dye (100 mg).and Performance Validation Kit	1	80-6406-80
115/230 V~Glass fluorometry cuvette, fluorescent grade	1	80-6227-44
Lamp replacement assembly.	1	80-6228-96
Optics replacement kit. Includes filter, glass cover, mirrors, and O-ring	1	80-6229-34
Lid replacement assembly. Includes lid, latch, spring, and mounting screw	1	80-6229-53

Capillary Adaptor

Capillary Adaptor Kit. (includes capillary tubes, 10-, 50-, and 100 µl 20 each.)	1	80-6227-63
Capillary tubes, 10 µl	100	80-6227-82
Capillary tubes, 50 µl	100	80-6228-20
Capillary tubes, 100 µl	100	80-6228-01

Dye and standards

Hoechst 33258 dye, 100 mg	1	80-6226-87
Calf thymus DNA standard	250 µg	80-6227-06
4-methylumbelliferone standard, 100 mg	1	80-6227-25
Performance Validation Kit	1	80-6252-52

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